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Sensitivity of labile carbon fractions to tillage and organic matter management and their potential as comprehensive soil quality indicators across pedoclimatic conditions in Europe

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ABSTRACT

Soil quality is defined as the capacity of the soil to perform multiple functions, and can be assessed by measuring soil chemical, physical and biological parameters. Among soil parameters, labile organic carbon is considered to have a primary role in many soil functions related to productivity and environmental resilience. Our study aimed at assessing the suitability of different labile carbon fractions, namely dissolved organic carbon (DOC), hydrophilic DOC (Hy-DOC), permanganate oxidizable carbon (POXC, also referred to as Active Carbon), hot water extractable carbon (HWEC) and particulate organic matter carbon (POMC) as soil quality indicators in agricultural systems. To do so, we tested their sensitivity to two agricultural management factors (tillage and organic matter input) in 10 European long-term field experiments (LTEs), and we assessed the correlation of the different labile carbon fractions with physical, chemical and biological soil quality indicators linked to soil functions. We found that reduced tillage and high organic matter input increase concentrations of labile carbon fractions in soil compared to conventional tillage and low organic matter addition, respectively. POXC and POMC were the most sensitive fractions to both tillage and fertilization across the 10 European LTEs. In addition, POXC was the labile carbon fraction most positively correlated with soil chemical (total organic carbon, total nitrogen, and cation exchange capacity), physical (water stable aggregates, water holding capacity, bulk density) and biological soil quality indicators (microbial biomass carbon and nitrogen, and soil respiration).

We conclude that POXC represents a labile carbon fraction sensitive to soil management and that is the most informative about total soil organic matter, nutrients, soil structure, and microbial pools and activity, parameters commonly used as indicators of various soil functions, such as C sequestration, nutrient cycling, soil structure formation and soil as a habitat for biodiversity. Moreover, POXC measurement is relatively cheap, fast and easy. Therefore, we suggest measuring POXC as the labile carbon fraction in soil quality assessment schemes in addition to other valuable soil quality indicators.

1. Introduction

Soil organic carbon (SOC) is one of the most widely used soil quality indicators together with pH and available P and K (Bünemann et al., 2018). It affects various soil chemical, physical and biological properties and plays a primary role in multiple soil functions in agricultural soils, such as nutrient cycling, soil aggregate formation, water retention and habitat provision for biodiversity (Reeves, 1997). Soil organic carbon also plays an important role in climate regulation, with the potential of increasing carbon sequestration, offsetting fossil-fuel emissions and counteracting yield reduction created by extreme weather events (Lal, 2004). Despite the importance of SOC, its depletion is one of the main threats for agricultural soils. Agricultural measures that are aimed at increasing SOC stocks are therefore becoming a priority worldwide. For example, the "4 per Mille" Initiative (https://www.4p1000.org/) aims at implementing soil management practices such as reduced tillage and the use of cover crops, which can effectively increase SOC stocks (Lal, 2016). Such soil practices have the potential

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to increase carbon stocks directly via the addition of organic material but also indirectly through promoting aggregate formation, thus improving soil structure (Deb et al., 2015).

Soil organic carbon consists of multiple compounds, from simple to more complex molecules which can have different stability (Deb et al., 2015). Since changes induced by soil practices are often difficult to detect by total SOC measurement (Haynes, 2005), measuring rapidly changing SOC pools, such as labile carbon pools, might be more informative to assess soil quality (Awale et al., 2017; Gregorich et al., 1994; Quanying et al., 2014; Wander, 2004).

Labile organic matter in soil mainly originates from the decomposition of plant and faunal biomass, root exudates, and deceased microbial biomass (Bolan et al., 2011). Labile carbon is the SOC pool which is directly available for microbial activity and, hence, is considered to be the primary energy source for microorganisms (Chantigny, 2003; Haynes, 2005). Addition of organic matter as fertilizer (Gattinger et al., 2012) and reduced tillage will likely increase labile organic carbon (Cooper et al., 2016). In addition, these practices have the potential to enhance carbon and nitrogen cycling as well as soil aggregation, which is one of the primary mechanisms through which organic carbon is sequestered in soil (Panettieri et al., 2015). Therefore, labile carbon has potential as an indicator of soil functions, in particular: nutrient cycling (measured e.g. by soil nutrient contents and C mineralization), soil aggregate formation (measured e.g. by water stable aggregates), carbon sequestration (typically derived from changes in total organic carbon content) and habitat provision for biodiversity (currently assessed by biological indicators such as microbial biomass and abundance of faunal groups).

Multiple labile carbon fractions have been defined in the last thirty years. They are discerned based on the nature of their fractionation methodology, which can be chemical, physical or biological (Haynes, 2005). Labile carbon fractions determined by chemical fractionation are extracted from the soil with different chemical compounds. Dissolved organic carbon (DOC) represents the organic carbon in the soil solution that is extracted with water and passes a mesh with a pore size of 0.45 µm. Hydrophilic DOC (Hy-DOC) represents the more bioavailable part of the DOC (Bolan et al., 2011). DOC and Hy-DOC are small, mainly soluble fractions of total organic carbon (TOC), primarily comprised of root and microbial exudates, products of hydrolysis and leachates from organic matter. Particularly Hy-DOC can turn over very rapidly, while DOC fractions can also adsorb to mineral surfaces (Leinemann et al., 2018; Lundquist et al., 1999). Labile carbon can also be extracted with hot water (hot water extractable carbon, HWEC), which generally has higher concentration in soil than DOC (Ghani et al., 2003). Permanganate oxidizable carbon (POXC, also referred to as Active Carbon), K_2SO_4 extractable C, and acid (H_2SO_4, HCl) hydrolysable C are based on the use of extractants other than water. Although the quantities of HWEC and POXC are similar and both fractions probably comprise carbon derived from dissolved organic matter and microbial biomass, they are most likely derived from different organic matter fractions. HWEC largely (45–60%) comprises carbohydrates and amides derived from soil microorganisms, enzymes, root exudates and lysates, while POXC contains also compounds like lignin and complex polysaccharides (Ghani et al., 2003; Haynes and Beare, 1997). HWEC is mainly present in the soil solution or loosely bound to soil minerals, and is prone to short-term seasonal variation (Leinweber et al., 1995). Physical fractionation by particle size or density determines particulate organic matter carbon (POMC) which consists mainly of partially decomposed organic residues (Haynes, 2005) and contains microbial biomass together with fresh plant residues and decomposing organic matter (Gregorich et al., 1994; Sequeira and Alley, 2011). Finally, microbial biomass carbon (MBC) and mineralizable C are also considered labile organic carbon fractions (also called biological fractions), and they are normally determined by soil fumigation and measurement of evolved CO_2 produced by microbial respiration in closed or open incubation systems (Haynes, 2005; Vance et al., 1987).

Many studies have used labile carbon to assess the impact of agricultural management and land use change on soil quality (Awale et al., 2017; Geraei et al., 2016; Ibrahim et al., 2013; Mirsky et al., 2008). In addition, previous studies also compared different labile carbon fractions for their sensitivity to management (Culman et al., 2012; Dou et al., 2008; Geraei et al., 2016). However, still remains unresolved which labile carbon fraction is the most sensitive to management and can be usefully related to soil functions, and as such be used as a sensitive soil quality indicator. Different fractions have been suggested as the most sensitive to soil management, and various methodologies and protocols have been applied, hampering comparisons between studies (Poeplau et al., 2018). Moreover, the linkage between labile carbon fractions and soil functions is often assumed and not established (Bünemann et al., 2018), and the generality of applying labile carbon fractions as soil quality indicators as well as the general application of harmonized methods for labile carbon fractions determination has never been assessed across different European pedoclimatic zones and agricultural management systems.

The general objective of this study was to facilitate the assessment of soil quality in agricultural systems by identifying a biochemical parameter that is sensitive to soil disturbance and linked with soil functions. The specific objective of our study was to assess the suitability of five different labile carbon fractions - dissolved organic carbon (DOC), hydrophilic DOC (Hy-DOC), permanganate oxidizable carbon (POXC), hot water extractable carbon (HWEC) and particulate organic matter carbon (POMC) - as soil quality indicators across different pedoclimatic zones. To do so, we tested the sensitivity of the labile carbon fractions to tillage and organic matter input in 10 European long-term field experiments. Monitoring of long-term field experiments is essential in soil science for the generalization of conclusions about the effects of specific soil management on soil quality and soil functions (Debreczeni and Körschens, 2003). We assessed the relationship of the different labile carbon fractions with physical, chemical and biological soil properties linked to soil functions, in particular nutrient cycling, carbon sequestration, soil aggregate formation and soil as a habitat for biodiversity. We hypothesised that labile carbon concentrations would increase with reduced tillage and high organic matter input, being more sensitive than TOC. Moreover, we expected that labile carbon fractions would be positively correlated to chemical, physical and biological soil properties currently used as indicators for nutrient cycling, soil organic carbon sequestration, soil aggregation and habitat provision.

2. Materials and methods

2.1. Experimental sites and management

Ten European long-term field experiments (LTEs) with a minimum duration of 5 years were selected (Fig. 1). Our selection covered different European climatic zones: Dfb and Dfc (continental climate with cold winters and warm summer without a dry season, or with cold winters and temperate summers without a dry season, respectively), Cfb and Csb (temperate climate with warm summer with or without dry season, respectively) and Bsk (arid cold steppe climate) (Köppen, 1918) (Fig. 2, Table S1). Also, we covered different soil types (Vertic Cambisol, Haplic Luvisol, Fluvisol, Gleyic Podzol, Eutric Gleysol, and Eutric Cambisol (WRB, 2014).

Each LTE had unique management characteristics, but the main agricultural practices studied can be simplified as tillage (T) and organic matter addition (OM) (Fig. 2, Table S1). The comparison of farming systems (organic or integrated vs. conventional) studied in three LTEs (CH3, ES4 and NL2) was allocated to the factor OM, even though the treatments differed in other aspects as well (e.g. pesticides input). For NL1, SL1, PT1 and HU1 the organic matter addition was categorised based on the type of organic matter addition (mineral or no organic matter addition vs. organic matter addition). The contrast in

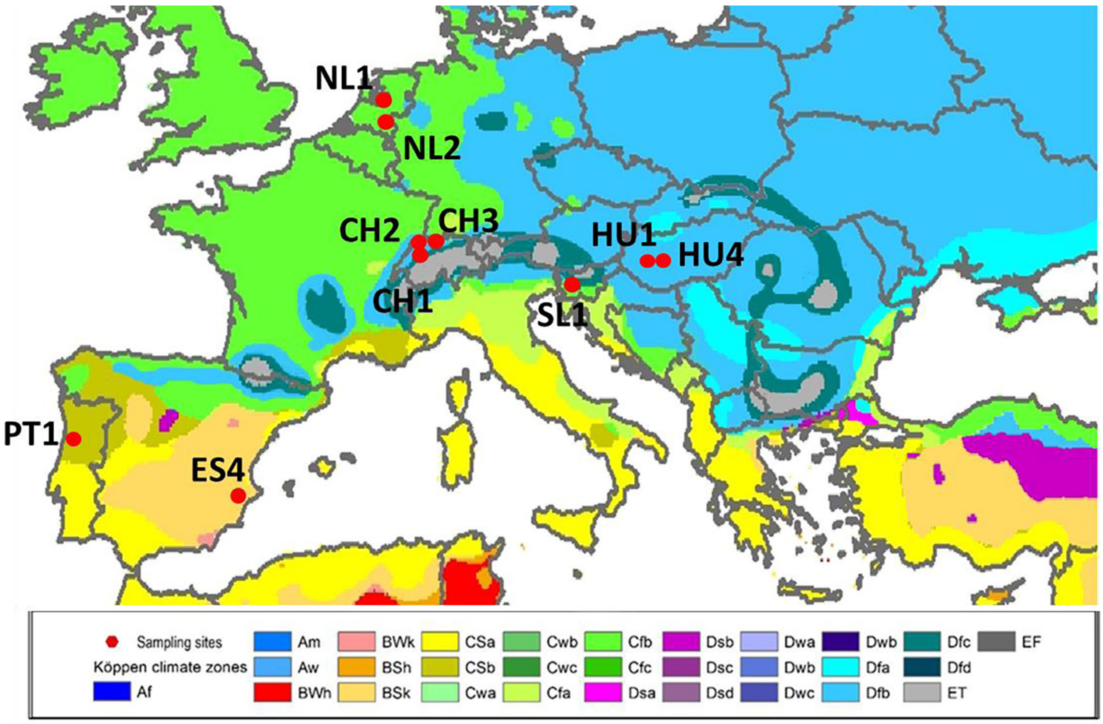

Fig. 1. Map showing the location of the 10 European long-term field experiments (LTE, here denoted with red dots and called "Sampling sites") used in the current study (Peel et al., 2007). The different colours on the map correspond to the Köppen climate zone classification. *CH1* Frick trial, CH2 Aesch trial, *CH3* DOK trial, *HU4* Keszthely trial, *HU1* Keszthely trial, *SL1* Tillorg trial, *NL2* De Peel trial, *NL1* Basis trial, *PT1* Vitichar trial, *ES4* Pago trial.

tillage was categorised as conventional tillage (ploughing to 20–25 cm depth, CT) versus reduced tillage (tillage to 0–10 cm, RT) and studied in six LTEs (CH1, CH2, HU4, NL1, NL2 and SL1). The level of OM addition was categorised as low organic matter input (LOW, no organic matter additions or only mineral fertilization) versus high organic matter input (HIGH, organic matter additions or organic matter additions with mineral fertilizer). At some sites, both treatment factors (i.e. T and OM) were implemented and at others only one of these (Fig. 2). The layout of the LTEs followed different designs, including complete randomized block and split plot design, and per treatment 3 or 4 replicates were present (Table S1). Most LTEs had arable crop rotations, but two LTEs (ES4 and PT1) in drier climates had grapes as permanent crops.

2.2. Sampling procedure and sample handling

In total, 167 soil samples were collected in spring 2016 before any major soil management was applied to the fields. Each sample comprised 20 soil cores randomly collected in the central area of the plot to avoid border effects. In the trials with tillage as management factor, samples were taken from two depths: 0–10 cm and 10–20 cm (Table S1). In the trials with organic matter input as the only management factor, samples were taken from the 0–20 cm layer. Shortly after collection, fresh soil samples were sent to Wageningen University (The Netherlands), Research Institute of Organic Agriculture (Frick, Switzerland), University of Trier (Germany) and University Miguel Hernandez (Alicante, Spain), and air-dried samples were sent to University of Ljubljana (Slovenia). Upon arrival, fresh samples were sieved at 5 mm and stored at 3 °C. The samples were used for measuring chemical, physical and biological parameters. All the analyses were performed within 6 months after sampling. A part of the samples was subsequently air-dried for POXC and POM-C analysis.

2.3. Chemical, physical and biological soil parameters

Various chemical, physical and biological soil parameters, selected to represent soil functions and general soil characteristics, were determined as follows. Total organic carbon (TOC) and total organic nitrogen (TON) were determined by elementary C and N analysis with combustion > 950° by a Vario Max Elemental Analyser. In case of calcareous soils, the samples were pre-treated with HCl to remove inorganic carbon. The pH was measured with a glass electrode WTW pH 538 in 0.01 M $CaCl_2$. Cation exchange capacity (CEC) was determined using a barium chloride solution buffered at pH 8.1. Plant available phosphorus (P_2O_5), plant available potassium (K_2O), and exchangeable magnesium, calcium, sodium and potassium (Mg^{2+}, Ca^{2+}, Na^+, K^+) were determined using ammonium-acetate extraction (van Reeuwijk, 2002). Available phosphorous-Olsen (P-Ol) was determined according to Olsen et al. (1954). These chemical parameters were measured as a *proxy* for the soil functions carbon sequestration and nutrient cycling.

Water-stable aggregates (WSA) were measured by a wet sieving method (Kemper and Koch, 1966) using an apparatus designed by Murer et al. (1993). Particle size distribution was determined by sieving and sedimentation (SIST ISO 11277:2011). Soluble salt and gypsum were removed and organic matter was destructed. Material between 0.063 and 2 mm was wet-sieved, while material < 0.063 mm was determined by sedimentation. Water holding capacity (water content at field capacity, pF 2.5) was calculated using the particle size distribution characteristics and the organic carbon content as described in Tóth et al. (2015). Water stable aggregates and water holding capacity were measured as a *proxy* for the soil functions soil structure formation and water retention.

Microbial biomass carbon (MBC) and nitrogen (MBN) were determined with the method of chloroform-fumigation extraction of Vance et al. (1987), using 0.01 M $CaCl_2$ as extractant. Concentrations of dissolved C and N in fumigated and non-fumigated subsamples were determined with a Shimadzu TOC Analyzer (V CPN E200V), and MBC and MBN were calculated as the difference between fumigated and non-fumigated subsamples, with conversion factors of 0.45 and 0.4 for incomplete extraction of microbial C and N, respectively (Vance et al., 1987). To assess basal soil respiration (SR), moist samples (approx. 60%

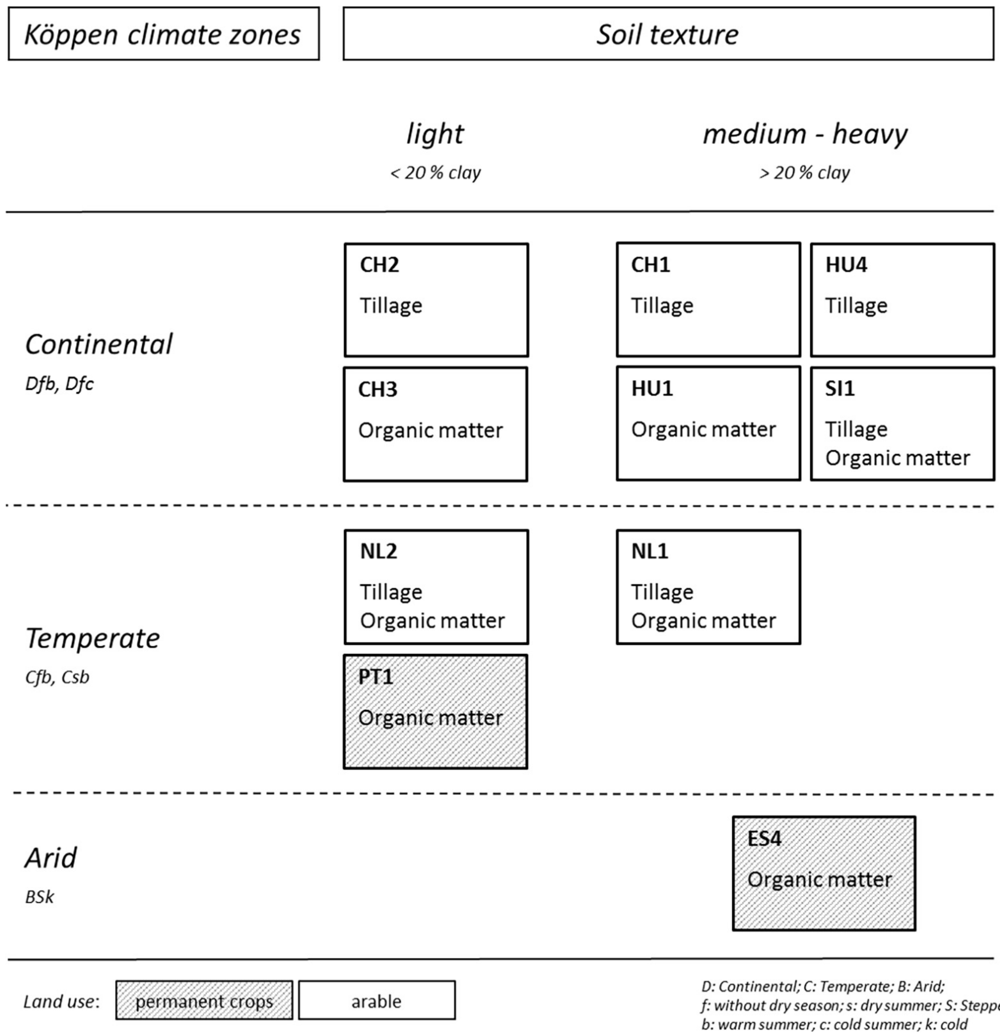

Fig. 2. Main pedoclimatic characteristics and management practices (categorised in tillage or organic matter input, or a combination of the two practices) of ten long-term field experiments analysed in the current study. *T* tillage, *OM* organic matter addition. *CH1* Frick trial, CH2 Aesch trial, *CH3* DOK trial, *HU4* Keszthely trial, *HU1* Keszthely trial, *SL1* Tillorg trial, *NL2* De Peel trial, *NL1* Basis trial, *PT1* Vitichar trial, *ES4* Pago trial. For detailed information about the experiments we refer to Table S1 in the supplementary materials.

of WHC) were incubated at 25 °C for 72 h in a thermostat bath where the bottles were connected to a respirometer (Micro-Oxymay, Columbus, OH, USA). The CO_2 rate was determined when it stabilized at 72 h from the beginning of the incubation. Metabolic quotient (qCO_2) and microbial quotient (qMic) were calculated as the ratio of soil respiration to microbial biomass carbon and the ratio of microbial biomass carbon to total organic carbon, respectively (Anderson and Domsch, 1990). Earthworms were collected in sampling plots of 30 × 30 cm with a mixed method consisting of hand sorting the top 30 cm and irritating with mustard solution (10 L per plot). The mustard solution comprised 6 g of dry powder mustard that was mixed with 1 L of water, and this solution was added to the excavated soil pit. In the lab, the earthworms were stored overnight at 15 °C in a jar with moist tissue, to allow them to void their gut. All individual earthworms were afterwards counted and weighed, and for the individuals that were damaged only the body parts containing the head were counted. Microbial biomass carbon and nitrogen, ecological indices and earthworm biomass and abundance were measured as *proxies* for the soil habitat function. Basal respiration was measures as a *proxy* for soil nutrient cycling.

The chemical and physical parameters were assessed at the University of Ljubljana, while microbial biomass was assessed at the University of Trier and basal soil respiration at the University Miguel Hernandez. Other physical and biological properties were assessed in the fields by the long-term field experiment owners. Soil bulk density (BD) was determined with calibrated sample cylinders of 100 cm^3 and special augers (Ø 0.05 m, Eijkelkamp, NL) that were used to take undisturbed soil samples in one or two layers, depending on the tillage treatment. The soil bulk density was calculated as follows:

$$Bulk\ density\,[g\ cm^{-3}] = \frac{dry\ weight\ [g]}{ring\ volume\ [cm^3]} \quad (1)$$

The measurement of plant residue decomposition was based on the decomposition of green tea or rooibos tea in bags, as described by Keuskamp et al. (2013). Briefly, per plot, four tea bags of each tea type were weighed and buried 8 cm deep. After approximately 90 days, the tea bags were recovered, dried for 48 h at 70 °C and weighed. In CH1 and CH2, fine material entered the tea bags and influenced the results. Therefore, to get a more precise estimation, the content of the tea bags was combusted at 550 °C and the final weight after combustion (which consisted only of soil particles) was subtracted from the content weight before combustion. Penetration resistance was determined using penetrometer loggers, with different instruments used by the different LTE owners. Per plot, 10 probes were made of which the results were averaged. The soil resistance pressure was measured until 50 cm depth for every 5 cm.

2.4. Labile carbon measurements

2.4.1. Dissolved organic carbon (DOC) and hydrophilic DOC (Hy-DOC)

Twenty g of field moist soil was used to extract dissolved organic carbon (DOC) as described in Van Agtmaal et al. (2017) and adapted as follows. Briefly, the samples were mixed with ultrapure water at a soil-to-solution ratio of 1:2 (dry wt/vol) in DOC-free polypropylene tubes, shaken for 1 h, centrifuged for 20 min at 3750 rpm and subsequently for 10 min at 10000 rpm. The samples were then filtered at 0.45 µm with cellulose acetate Whatman® Puradisc membrane filters to obtain total DOC. Filters were pre-rinsed with ultrapure water and flushed with air to avoid any release of DOC during filtration. A fraction of the DOC obtained was subsequently acidified to pH 1 with 6 M HCl to extract the hydrophilic part of the DOC (Hy-DOC) using a simplified DOC fractionation scheme adapted from Van Zomeren and Comans (2007). During the fractionation the hydrophobic components of DOC present in solution (humic and fulvic acid, and hydrophobic neutrals) bind to an added insoluble polymeric adsorbent (Supelite™ DAX-8, Sigma-Aldrich). Only the hydrophilic part of the DOC remains in solution not binding to the resin and can subsequently be quantified. Briefly, the DAX-8 resin was added to the acidified solutions to reach a ratio of 1:5 (wt/vol). The solution was then shaken horizontally for one hour at 180 rpm, centrifuged for 5 min at 3750 rpm, and the supernatant containing the hydrophilic part of DOC was collected. The total carbon (C) concentration of both the DOC solution and the supernatant was determined on a TOC-5050A analyser (Shimadzu Corporation, Kyoto, Japan). DOC and Hy-DOC fractions were further analysed for specific ultraviolet absorbance (SUVA) to assess their aromaticity (Weishaar et al., 2003). To this end, 1.5 ml extracted DOC and Hy-DOC from each sample were analysed with a spectrophotometer (Genesys 10S UV–VIS, Thermo Fisher Scientific Inc., Waltham MA, USA) and ultrapure water was used as a blank. The aromaticity of the two fractions expressed by the SUVA ($L\,g\,C^{-1}\,cm^{-1}$) at 254 nm was calculated as described in Weishaar et al. (2003) and adapted by Amery et al. (2008):

$$SUVA = \frac{A_{254} * 1000}{b * [DOC]} \tag{2}$$

where A_{254} is absorbance at 254 nm (dimensionless), b is the path length (cm) and DOC (or Hy-DOC) is the dissolved organic carbon concentration ($mg\ L^1$) of the solution.

2.4.2. Hot water extractable carbon (HWEC)

Hot water extractable carbon (HWEC) was determined according to the methodology of Ghani et al. (2003). Briefly, 4 g of soil was mixed with 30 ml of deionized water in a 50 ml polypropylene centrifuge tube. The tube was shaken horizontally for 30 min at 150 rpm and centrifuged for 20 min at 3500 rpm. The supernatant obtained at this stage (water-soluble carbon) was discarded. An additional 30 ml of deionized water was added to the sediments remaining in the tube and the tube was shaken for 10 s to suspend the soil in the water. Subsequently, the closed tubes were placed in an oven at 80 °C for 16 h. After this step, the tubes were shaken for 10 s in a vortex shaker and centrifuged for 20 min at 3500 rpm, and additionally for 10 min at 10000 rpm if necessary (to bring down the solid). The supernatants were filtered using 0.45 µm cellulose nitrate filter membranes and total carbon was determined on a TOC-5050A analyser (Shimadzu Corporation, Kyoto, Japan).

2.4.3. Permanganate oxidizable carbon (POXC)

The permanganate oxidizable carbon (POXC, also referred to as Active Carbon) was extracted and analysed following the procedure of Weil et al. (2003) modified as follows. Briefly, 2.5 g of air-dried soil was weighed into a polypropylene tube and 18 ml of demineralized water and 2 ml of 0.2 M K_2MnO_4 was added. The tube was shaken for 2 min at 120 rpm and thereafter left undisturbed on a lab bench for 8 min to continue the oxidation reaction. Subsequently, 0.5 ml of solution was taken from the tube and placed in another tube with 49.5 ml of demineralized water, allowing the reaction to stop. The absorbance of each sample at 550 nm (Abs) was determined using a GENESYS 10S UV–VIS Spectrophotometer. Permanganate oxidizable carbon was calculated according to Weil et al. (2003):

$$POXC\,(mg\ kg^{-1}) = [0.02\ molL^{-1} - (a + b * \ \mathrm{Abs})] * (9000\ mg\ \mathrm{C}\ mol^{-1})\ (0.02\ L\ solution\ Wt^{-1}) \tag{3}$$

where $0.02\ mol\ L^{-1}$ is the concentration of the K_2MnO_4 solution, a is the intercept and b is the slope of the standard calibration curve, 9000 mg is the amount of carbon oxidized by 1 mol of MnO_4 changing from Mn^{+7} to Mn^{+4}, 0.02 L is the volume of the K_2MnO_4 reacting with the samples, and Wt is the mass of soil in kg used for the reaction.

2.4.4. Carbon from particulate organic matter (POMC)

The particulate organic matter was characterized as reported by Wyngaard et al. (2016) modified from Salas et al. (2003). Briefly, 10 g of dry soil samples was shaken for 15 h with 30 ml of 1 M NaCl on a horizontal shaker. Subsequently the suspension was wet-sieved through a 53 µm sieve. The material on top of the sieve was transferred to a crucible and dried overnight at 105°C. The samples were weighted (M1) and placed in a furnace at 550°C for 4 h before weighing them again (M2). The POM was calculated by loss of ignition, i.e. as the weight loss during combustion at 550 °C in the muffle furnace. The POMC was calculated dividing POM values for 1.724, assuming that the percentage of organic carbon in the POM was 58%. This conversion factor has been criticized and might not be completely correct, but for the purpose of this study we needed an approximation and small differences in the C content of POM will not compromise the use of the calculated POMC (Pribyl, 2010).

2.4.5. Labile carbon and TOC stocks

Labile carbon and TOC stocks were calculated in the different layers taken into account in the study as:

$$C\ stock\ (Mg\ C\ ha^{-1}) = \left[BD\left(\frac{g}{cm^3}\right) * Soil\ depth\ (cm) * Labile\ C\ concentration\,(g\ kg^{-1})\right] * 100 \tag{4}$$

where *BD* is the bulk density expressed in $g\,cm^{-3}$, *soil depth* is the soil layer sampled, and *Labile C concentration* is the concentration of labile carbon measured in $g\,kg^{-1}$. For the LTEs where the two layers were sampled, C stocks were calculated in the two layers separately and then added to obtain the value of the stocks in the 0–20 cm layer.

2.5. Statistical analysis

All statistical calculations were carried out using R version 3.3.2 (R Development Core Team, 2013). For the linear mixed effects model, the packages *nlme* (Pinheiro et al., 2018) and *emmeans* (Lenth et al., 2018) were used, while for the correlation analysis the packages *car* (Fox and Weisberg, 2011) and *stats* were used.

The effects of soil management on the labile carbon fractions (presented either in $mg\ kg^{-1}$, percentage of TOC or as C stocks) per site across the 10 European long-term field experiments were assessed using linear mixed effects models. Mixed models were used to take into account the possible correlations introduced by the multi-site field experiments and to generalize the effect of the management practices across the different LTEs (Bradford et al., 2013; Lucas and Weil, 2012). The tillage and/or the soil organic matter addition and, if distinguished, the layer, their two-way and, if applicable, three-way interactions were used as fixed factors. Random effects of trials, blocks, main plots and subplots were introduced in the models to represent the experimental designs of the different trials. The effect of the soil pedoclimatic zone was not included in the fixed part of the model because we were interested in the management effects across the pedoclimatic zone. Three separate linear mixed effect models were applied to three subsets of the LTEs:

1 – Tillage model. The primary factor of interest in this analysis was tillage, followed by OM. To assess the effect of tillage and layer, the LTEs CH1, CH2, NL1, NL2, SL1 and HU4 were used and the analysis was performed on data from both layers (0–10 cm and 10–20 cm). For these trials, the stratification ratio for the labile carbon fractions in RT and CT was calculated and analysed in the linear mixed effect model according to Franzluebbers (2002):

$$Stratification\ Ratio = \frac{Labile\ carbon\ mg\ kg^{-1}\ in\ 0-10\ cm}{Labile\ carbon\ mg\ kg^{-1}\ in\ 10-20\ cm} \tag{5}$$

2 – OM model. The primary factor of interest in this analysis was the OM addition, followed by tillage. For this analysis, the LTEs analysed were NL1, NL2, SL1, CH3, HU1, PT1, ES4 and the 0–20 cm layer was used. In the LTEs in which the two layers were sampled separately, the value of the 0–20 cm layer was taken as the average of the 0–10 and the 10–20 layers.

3- Stocks model. The factors of interest in this analysis were tillage and OM addition. For the analysis of the labile carbon stocks (Mg ha^{-1}) in the 0–20 layer, all ten trials were used.

The effect of agricultural management and the layer, if applicable, on the labile carbon concentrations was assessed in each long-term field experiment with linear mixed effect models.

The effects of tillage and fertilization and their interaction on the labile carbon fractions were addressed by performing F-tests (using the function *anova*) for the fitted linear mixed effect model. For all the studied variables, the model assumptions of normality and homogeneity of variances of the residuals were checked both visually (plotting sample quantiles versus theoretical quantiles and residuals versus fitted values) and with the Shapiro-Wilk and Levene's tests (Zuur, 2009). Variables whose residuals did not meet these assumptions were log-transformed or square root-transformed and then used for analysis. If the transformation did not meet the criteria, the function *weights* was used in the linear mixed model effect formula to take into account the non-homogeneous variance structure introduced by the factors studied (Zuur, 2009). The function *emmeans* was used to estimate the marginal means and Tukey HSD post-hoc tests were used to assess significant differences between treatments when the F-tests indicated statistically significant effects. All test results were considered statistically significant at $p \leq 0.05$.

Spearman's rank correlation was used to examine the relationships between labile carbon fractions and biological, physical and chemical soil quality parameters across the LTEs. Correlation analysis was done on log-transformed or square root-transformed variables. The relationship between labile carbon fractions and soil parameters was validated using partial correlations, correcting for variation caused by the intrinsic differences of the LTEs (pedoclimatic zones). Partial correlations can, in fact, remove the effect of a variable (in this case the LTE) which might control the observed relationship between two variables. When partial correlations are applied, the relationship between two variables is independent from the controlling variable. In addition, we calculated the average correlation coefficients between the labile carbon fractions and the three indicators' groups (chemical, physical and biological), and the overall average correlation coefficient with the entire set of soil parameters.

3. Results

The concentrations of DOC, Hy-DOC, POXC, HWEC and POMC differed widely between the LTEs, but their order of magnitude was consistent across the 10 LTEs (Fig. 3, Table S2).

Hy-DOC was the least abundant fraction per unit of soil or per unit of total organic C (0.004–0.050% of TOC), followed by DOC (0.06–0.40% of TOC), POXC (1.45–4.32% of TOC), HWEC (1.0–6.0% of TOC) and finally POMC (8–52% of TOC). In comparison, microbial biomass carbon was intermediate between DOC, POXC and HWEC (0.12–2.84% of TOC). POXC and HWEC were similar in their concentration and total share in the TOC. Among the labile carbon fractions and across all the LTEs, the fraction with the lowest coefficient of variation (calculated using all data points) was POXC (32%), followed by DOC (42%), Hy-DOC (43%), HWEC (51%) and POMC (52%). Most labile carbon fractions had lower concentrations in the lower than in the upper layer, with the exception of DOC, which was often higher in the lower layer. The LTEs HU1 and PT1 had the lowest concentrations of labile carbon across the different fractions. We did not find specific LTEs that had consistently higher or lower labile carbon fractions expressed as percentage of TOC. Table S3 shows the results of the analysis of the effect of the soil management on the labile carbon fractions for each of the LTEs.

3.1. Effect of tillage on the labile carbon fractions

The labile carbon fractions differed in their sensitivity to tillage (Table 1).

Looking at the *F* statistics, POXC and POMC (mg kg^{-1} soil) were the fractions most sensitive to tillage. However, there was a significant interaction between tillage and layer for POXC, HWEC and POM, since concentrations of these three fractions were higher under RT than CT in the upper layer only (Fig. 4).

Accordingly, we found higher values of stratification ratio in RT than CT with both LOW and HIGH organic matter input for POXC, HWEC and POMC (Table S4). For Hy-DOC and DOC, the stratification ratio was higher in RT, but only with low organic matter input. For TOC only a trend ($p = 0.057$) of higher values under RT was found. When expressed as percentage of TOC, the labile carbon fractions were not affected by the tillage treatment but only by the layer (Table S5 and Figure S1). In the same way, the aromaticity of the DOC and Hy-DOC as measured by $SUVA_{254}$ was not affected by the tillage treatments across the sites, but DOC SUVA was affected by the layer (Table S6 and Figure S2). The significant interaction that we found means that in the reduced tillage plots, the aromaticity of DOC was greater in the lower than in the upper layer.

3.2. Effect of OM addition on the labile carbon fractions

All labile carbon fractions were significantly higher in high OM compared to low OM input trials (Table 2).

In the analyses, the type of tillage applied to the plots was also taken into account. POXC, HWEC and POMC (mg kg^{-1}) were significantly increased in RT compared to CT plots. POXC, Hy-DOC and POMC (mg kg^{-1}) were the more sensitive labile carbon fractions (taking into account the *F* statistics). When labile carbon fractions were expressed as percentage of total organic carbon, only Hy-DOC, POXC and POMC were significantly higher in the high OM compared to low OM input trials (Table S7). In addition, the positive effect exerted by the high organic matter input on POXC was stronger in trials with CT. Aromaticity of DOC and Hy-DOC as measured by $SUVA_{254}$ was not affected by organic matter addition across all the sites (Table S8).

3.3. Effect of tillage and OM addition on the labile carbon stocks across the 10 LTEs

Reduced tillage and high OM input both significantly increased labile carbon stocks expressed in Mg ha^{-1}, i.e. stocks of all the fractions (Table 3).

POXC and POMC were affected most by the two management factors, as indicated by higher *F* statistics. The TOC stock was less sensitive than the stocks of labile C fractions, being affected neither by organic matter addition nor by tillage.

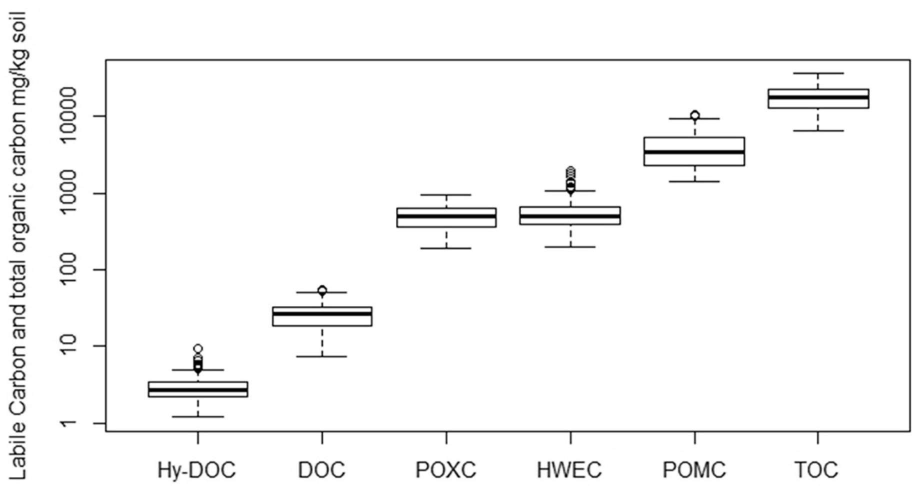

Fig. 3. Box plot of the concentrations of hydrophylic dissolved organic carbon (Hy-DOC), dissolved organic carbon (DOC), permanganate oxidizable carbon (POXC), hot water extractable carbon (HWEC), particulate organic matter carbon (POMC), and total organic carbon (TOC) in mg kg^{-1} soil across all 10 LTEs (n = 167). We report a logarithmic y-axis. The boxes represent the values between the 25th and the 75th percentiles, the thin lines represent the minimum and the maximum values and the thick line is the median. The open dots are outliers.

Table 1
Effects of tillage (CT vs. RT), organic matter addition (LOW vs. HIGH), and layer (0–10 cm and 10–20 cm) on the labile carbon fractions for the tillage trials as analysed with mixed linear effects models (number of observations = 120). In the upper part of the table the estimated means and 95% confidence intervals (in parentheses) of Hy-DOC, DOC (mg kg^{-1} soil), POXC, HWEC, POMC and TOC (g kg^{-1} soil) under tillage and organic matter (OM) management are reported. Different letters following means have to be read per columns and per layer; they show treatments which are significantly different ($p \leq 0.05$) according to Tukey post-hoc tests for the three way interactions. In the lower part of the table, F statistics and *p*-values (values ≤ 0.05 are given in bold) for the main factors and their interactions are reported.

		Hy-DOC	DOC	POXC	HWEC	POMC	TOC
Layer 0–10 cm		(mg kg^{-1} soil)		(g kg^{-1} soil)			
CT- LOW		2.6ab (1.97–3.38)	22.1a (14.6–31.2)	0.50 (0.39–0.66)	0.53 (0.46–0.96)	3.7 (1.95–5.44)	20 (13.9–25.7)
RT- LOW		3.2b (2.43–4.17)	27.7ab (19.2–37.8)	0.63 (0.48–0.77)	0.72 (0.50–1.03)	4.9 (3.15–6.74)	21 (15.6–27.3)
CT- HIGH		3.2ab (2.41–4.66)	26.4ab (17.8–36.7)	0.55 (0.42–0.70)	0.66 (0.37–0.75)	4.4 (2.62–6.25)	23 (17.1–28.7)
RT- HIGH		3.1ab (2.36–4.17)	25.6ab (17.2–35.7)	0.60 (0.47–0.77)	0.71 (0.49–1.03)	5.1 (3.13–7.09)	24 (17.4–30.4)
Layer 10–20 cm							
CT- LOW		2.6ab (2.01–3.49)	27.5b (19.0–37.5)	0.53 (0.42–0.68)	0.53 (0.37–0.76)	3.5 (1.80–5.29)	19 (13.4–24.7)
RT- LOW		2.4a (1.85–3.18)	25.5ab (17.3–35.2)	0.51 (0.39–0.65)	0.48 (0.33–0.68)	3.3 (1.53–5.13)	19 (13.5–25.1)
CT- HIGH		3.5ab (2.61–4.66)	27.9ab (19.1–38.6)	0.57 (0.44–0.73)	0.57 (0.39–0.82)	4.3 (2.51–6.14)	19 (12.8–25.1)
RT- HIGH		3.5b (2.63–4.71)	29.4ab (20.3–40.3)	0.54 (0.43–0.71)	0.52 (0.36–0.76)	4.0 (2.06–6.02)	18 (12.6–24.3)
Tillage (T)	F	0.33	0.45	3.81	0.95	7.29	0.09
	p	0.56	0.64	**0.04**	0.33	**0.01**	0.75
OM	F	10.16	0.82	2.72	2.38	11.25	6.7
	p	**0.002**	0.37	0.09	0.13	**0.003**	**0.01**
Layer (L)	F	0.12	5.5	3.81	22.7	15.4	44
	p	0.72	**0.02**	**0.04**	**< 0.0001**	**0.0002**	**< 0.0001**
T X OM	F	0.42	0.27	1.73	1.4	0.7	2.7
	p	0.51	0.60	0.19	0.24	0.38	0.11
T X L	F	3.33	2.25	19.18	9.5	28.3	2.2
	p	0.07	0.13	**< 0.0001**	**0.003**	**< 0.0001**	0.14
OM X L	F	9.57	0.28	1.03	0.09	0.28	12.5
	p	**0.003**	0.59	0.31	0.76	0.59	**0.008**
T X OM X L	F	5.15	7.46	2.67	1.91	0.58	0.009
	p	**0.02**	**0.008**	0.10	0.17	0.44	0.92

Hy-DOC hydrophilic dissolved organic carbon, *DOC* dissolved organic carbon, *POXC* permanganate oxidizable carbon, *HWEC* hot water extractable carbon, *POMC* particulate organic matter carbon, *TOC* total organic carbon, *LOW* low organic matter input, *HIGH* high organic matter input, *CT* conventional tillage, *RT* reduced tillage.

3.4. Correlation of labile carbon fractions with other soil quality parameters

We tested the bivariate relationships between the labile carbon fractions and soil chemical, physical and biological indicators across both soil layers where applicable. In addition to bivariate correlations, we validated the obtained relationships by carrying out partial correlations where we corrected for the variation caused by the LTE (Table 4).

POXC was the labile C fraction that was most significantly (*p*-values), and strongly (Spearman's correlation coefficients, ρ), correlated with the soil chemical, physical and biological indicators related to nutrient cycling, soil structure and biodiversity both in the bivariate (Table S9) and the partial (Table 4) correlations. Moreover, POXC proved to be highly positively correlated ($p < 0.0001$) with Hy-DOC (ρ = 0.59), DOC (ρ = 0.41), HWEC (ρ = 0.60) and POMC (ρ = 0.70) (Table 5 residuals and S10 original data). The other carbon fractions were correlated with each other but not so strongly, with the only exception of strong positive correlations between Hy-DOC and (in addition to POXC) DOC, HWEC, and POMC ($p < 0.0001$, ρ = 0.41; $p < 0.001$, ρ = 0.41; $p < 0.0001$, ρ = 0.50, respectively) (Table 5).

4. Discussion

The ranges of labile organic C fractions measured in this study were in accordance with those reported previously (Benbi et al., 2015; Lucas and Weil, 2012; Margenot et al., 2017). Hy-DOC accounted for the

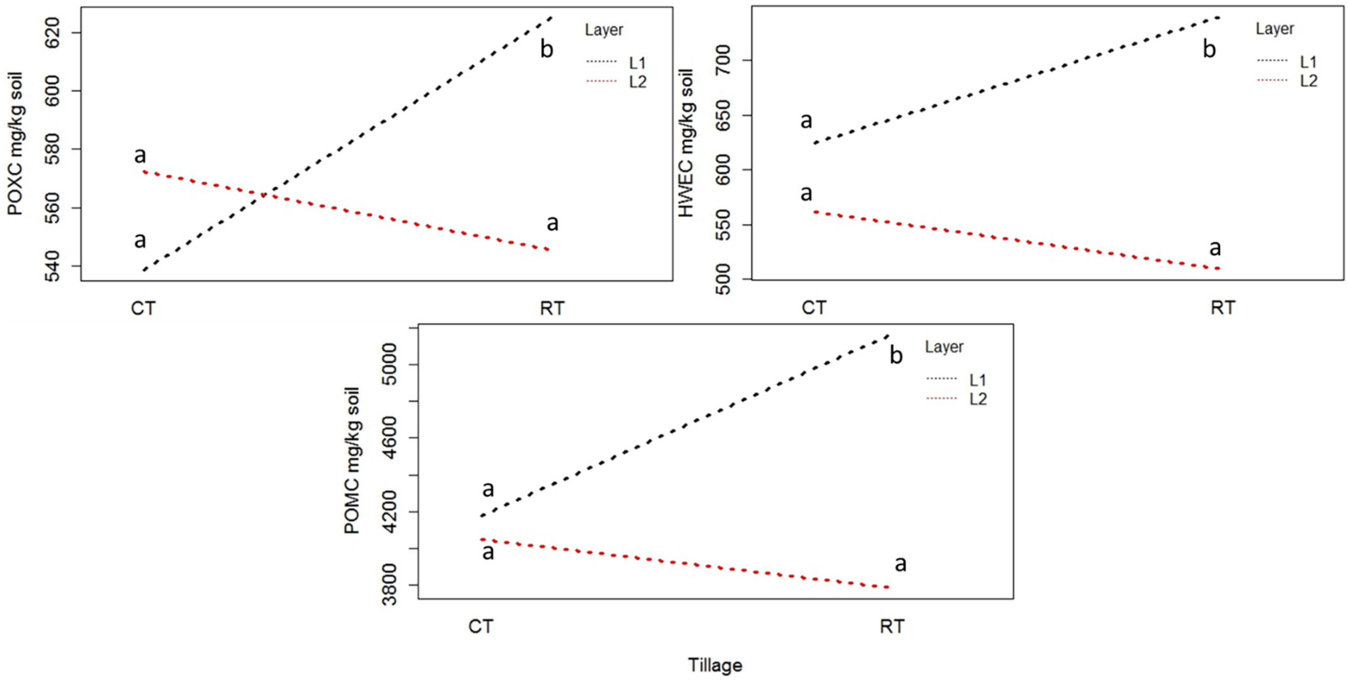

Fig. 4. Interaction plots showing the 2-way interaction between tillage and layer (L1, L2) for the variables POXC, HWEC and POMC expressed in mg kg^{-1} soil for the tillage trials as analysed with mixed linear effects models (number of observations = 120). Different letters show the treatments which are significantly different ($p \le 0.05$) according to Tukey post-hoc test for the 2-way interaction. *POXC* permanganate oxidizable carbon; *HWEC* hot water extractable carbon*; POMC* particulate organic matter carbon; *CT* conventional tillage; *RT* reduced tillage; *L1* 0–10 cm and *L2* 10–20 cm soil depth. Note that the y-axes do not start at zero.

smallest part of TOC, followed by DOC, POXC, HWEC and POMC. These results demonstrate that the different methodologies extract different parts of the total organic carbon.

4.1. Effect of tillage on the six labile carbon fractions

In the analysis across the six tillage trials, POXC, HWEC and POMC in the upper soil layer were higher in RT than in CT (Fig. 3). Several studies reported that RT increases the concentration of soil labile carbon compared to CT (Aziz et al., 2013; Liu et al., 2014; Neogi et al., 2014). Tillage disrupts macro- and micro-aggregates, increases soil temperature and aeration and releases soil organic matter which was protected in these physical structures (Six et al., 1999). Soil organic matter can thus become more available to soil organisms, increasing CO_2 emissions and decreasing the labile fractions. This phenomenon is fostered by the greater transfer of residues, mineral fertilizers and organic amendments to deeper soil layers during conventional tillage. In reduced tillage, on the other hand, the labile carbon protected in aggregates can accumulate in the soil (Jastrow et al., 2006). Under these conditions, microbial biomass and activity can be favoured, increasing the production of enzymes which can increase soil labile C fractions (Melero et al., 2011).

Our study corroborates previous findings which also detected HWEC, POXC and POMC as the labile C fractions that are most sensitive to tillage (Chen et al., 2009; Ćirić et al., 2016), in particular POXC and POMC (Culman et al., 2012; Plaza-Bonilla et al., 2014; Prasad et al., 2016). These fractions were more sensitive than TOC, which confirms the early warning capacity of labile carbon in indicating soil quality

Table 2
Effects of organic matter (OM) addition (LOW vs. HIGH) and tillage (CT vs. RT) on the labile carbon fractions for the OM input trials in the 0–20 cm layer as analysed with mixed linear effects models (number of observations = 119). In trials where the 0–10 cm and the 10–20 cm layers were sampled separately, we averaged the C values over the two layers. In the upper part of the table the estimated means and 95% confidence intervals (in parentheses) of Hy-DOC, DOC (mg kg^{-1} soil), POXC, HWEC, POMC and TOC (g kg^{-1} soil) under OM and tillage management are reported. In the lower part of the table F statistics and *p*-values (values ≤ 0.05 are given in bold) for the main factors and their interactions are reported.

		Hy-DOC	DOC	POXC	HWEC	POMC	TOC
Layer 0–20 cm		(mg kg^{-1} soil)			(g kg^{-1} soil)		
LOW-CT		2.2 (1.71–2.91)	23.4 (14.9–33.7)	0.40 (0.26–0.53)	0.38 (0.28–0.53)	2.4 (1.80–3.22)	16.2 (11.6–20.8)
LOW-RT		2.2 (1.63–3.00)	24.6 (15.1–36.5)	0.44 (0.31–0.58)	0.46 (0.32–0.66)	2.8 (2.07–3.86)	15.5 (10.8–20.2)
HIGH-CT		2.9 (2.24–3.78)	27.4 (18.3–38.4)	0.47 (0.34–0.60)	0.47 (0.34–0.64)	2.8 (2.09–3.74)	17 (12.4–21.6)
HIGH-RT		3.0 (2.27–4.05)	27.8 (18.2–39.5)	0.50 (0.37–0.63)	0.53 (0.37–0.74)	3.1 (2.27–4.17)	16.8 (12.1–21.3)
OM	F	35.1	8.9	45.0	12.0	18.5	12.6
	p	**< 0.0001**	**0.006**	**< 0.0001**	**0.0002**	**0.0002**	**0.001**
Tillage (T)	F	0.05	0.02	6.5	3.9	5.8	1.13
	p	0.82	0.89	**0.02**	**0.05**	**0.02**	0.29
T X OM	F	0.16	0.01	0.70	0.22	1.18	1.18
	p	0.68	0.93	0.39	0.64	0.28	0.28

Hy-DOC hydrophilic dissolved organic carbon, *DOC* dissolved organic carbon, *POXC* permanganate oxidizable carbon*, HWEC* hot water extractable carbon, *POMC* particulate organic matter carbon*, TOC* total organic carbon, *LOW* low organic matter input, *HIGH* high organic matter input, *CT* conventional tillage, *RT* reduced tillage.

Table 3
Effect of organic matter OM addition (LOW vs. HIGH) and tillage (CT vs. RT) on the labile carbon stocks in the soil layer 0–20 cm expressed in Mg C ha^{-1} for all the trials as analysed with mixed linear effects models (number of observations = 101). In the upper part of the table the estimated means and 95% confidence intervals (in parenthesis) of the labile carbon fractions and TOC in organic matter and tillage management are reported. The lower part of the table shows F statistics and *p*-values (values ≤ 0.05 are given in bold) for the main factors and their interactions.

		Hy-DOC	DOC	POXC	HWEC	POMC	TOC
Layer 0–20 cm		(Mg C ha^{-1})					
LOW-CT		0.009 (0.006–0.012)	0.071 (0.05–0.09)	1.42 (1.05–1.91)	1.77 (1.14–2.39)	11.40 (6.74–16.10)	83.6 (44.03–123.2
LOW-RT		0.012 (0.009–0.015)	0.093 (0.06–0.12)	1.73 (1.24–2.29)	2.05 (1.39–2.76)	13.55 (8.77–18.33)	84.5 (44.80–124.3)
HIGH-CT		0.012 (0.009–0.014)	0.089 (0.07–0.12)	1.70 (1.28–2.33)	2.09 (1.53–2.81)	14.09 (9.38–18.79)	84.4 (44.79–124.0)
HIGH-RT		0.013 (0.010–0.016)	0.103 (0.07–0.13)	1.87 (1.37–2.55)	2.25 (1.61–2.98)	16.09 (11.31–20.87)	82.3 (42.52–122.0)
OM	F	13.4	12.7	32.0	14.3	29.4	0.28
	p	**0.0009**	**0.001**	< **0.0001**	**0.0007**	< **0.0001**	0.59
Tillage (T)	F	7.95	5.32	10.7	6.87	12.8	0.17
	p	**0.008**	**0.027**	**0.002**	**0.01**	**0.001**	0.67
T X OM	F	3.72	0.84	2.38	0.54	0.01	0.64
	p	0.06	0.36	0.13	0.46	0.89	0.43

Hy-DOC hydrophilic dissolved organic carbon, *DOC* dissolved organic carbon, *POXC* permanganate oxidizable carbon, *HWEC* hot water extractable carbon, *POMC* particulate organic matter carbon, *TOC* total organic carbon, *LOW* low organic matter input, *HIGH* high organic matter input, *CT* conventional tillage, *RT* reduced tillage.

disruption due to agricultural practices.

Dissolved organic carbon and Hy-DOC were less sensitive to tillage. Dissolved organic carbon (and consequently the most soluble Hy-DOC fraction) is very much dependent on environmental conditions (i.e. temperature and precipitation), and short-term management (Federici et al., 2017; Mouloubou et al., 2016; Soon et al., 2007). Moreover, in spring, which coincided with our sampling time, the level of DOC is the lowest throughout the year (Haynes, 2005; Schiedung et al., 2017).

Table 4
Partial correlation coefficients (ρ) between the labile organic carbon fractions expressed in mg kg^{-1} soil (Hy-DOC, DOC, POXC, HWEC and POMC) and % (TOC) and various soil chemical, physical and biological indicators used as dependent variable, corrected for the long term field experiments (LTEs). The number of samples used in the analyses was 167, but 101 for earthworm number, and earthworms biomass. In the table also the average correlation coefficients for each indicator group (chemical, physical and biological indicators) is reported, in addition to the overall average correlation coefficient (calculated across all the indicators).

	Hy-DOC		DOC		POXC		HWEC		POMC		TOC	
Chemical indicators												
TOC	0.44	***	0.33	***	0.69	***	0.52	***	0.68	***	1	
TON	0.54	***	0.42	***	0.73	***	0.57	***	0.63	***	0.79	***
CEC	0.18	*	0.27	**	0.43	***	0.24	*	0.23	*	0.35	***
C/N	−0.30	***	−0.39	***	−0.54	***	−0.36	***	−0.21	*	−0.26	**
pH	0.06	–	−0.24	*	0.06	–	0.03	–	0.13	–	0.10	–
P	0.24	*	0.08	–	0.29	**	0.27	**	0.27	**	0.36	***
P Olsen	0.18	*	0.15	*	0.22	*	0.29	**	0.28	**	0.33	***
Mg	0.16	*	0.21	*	0.45	***	0.22	*	0.21	*	0.33	***
Ca	0.24	*	−0.003	–	0.19	*	0.15	*	0.26	**	0.27	**
K	0.16	*	0.15	*	0.40	***	0.29	**	0.33	***	0.50	***
Na	0.15	*	0.11	–	0.02	–	−0.05	–	0.01	–	0.01	–
Average chemical	0.24		0.21		0.36		0.27		0.29		0.33	
Physical indicators												
WSA	0.30	**	0.32	***	0.53	***	0.35	***	0.35	***	0.44	***
WHC	0.19	*	0.19	*	0.30	**	0.28	**	0.25	*	0.49	***
BD	−0.10	–	−0.09	–	−0.28	**	−0.25	*	−0.38	***	−0.31	***
Sand	0.01	–	−0.21	*	0.01	–	−0.02	–	0.07	–	−0.01	–
Silt	0.14	–	0.13	–	0.05	–	0.08	–	0.09	–	0.09	–
Clay	−0.03	–	0.04	–	0.04	–	−0.02	–	−0.13	–	0.03	–
WC	0.20	*	0.20	*	0.24	*	0.12	–	0.32	***	0.29	**
Average physical	0.14		0.17		0.21		0.16		0.23		0.24	
Biological indicators												
MBC	0.40	***	0.13	–	0.59	***	0.52	***	0.53	***	0.54	***
MBN	0.28	**	0.16	*	0.47	***	0.41	***	0.38	***	0.32	***
SR	0.28	**	0.05	–	0.46	***	0.44	***	0.48	***	0.24	*
qCO_2	−0.07	–	−0.06	–	−0.15	–	−0.08	–	−0.11	–	−0.37	***
qMic	0.20	*	−0.06	–	0.26	**	0.30	***	0.20	*	0.01	–
Earthworm numbers	0.06	–	−0.16	–	0.07	–	0.02	–	−0.0003	–	−0.07	–
Earthworm biomass	0.05	–	−0.10	–	0.04	–	0.07	–	−0.15	–	−0.18	–
Decomposition	−0.12	–	−0.20	*	−0.34	**	−0.34	**	−0.27	*	−0.23	*
Average biological	0.18		0.11		0.30		0.27		0.26		0.24	
Average overall indicators	0.19		0.17		0.30		0.24		0.27		0.28	

Hy-DOC hydrophilic dissolved organic carbon, *DOC* dissolved organic carbon, *POXC* permanganate oxidizable carbon, HWEC hot water extractable carbon, POMC particulate organic matter carbon, TOC total organic carbon, TON total nitrogen, CEC cation exchange capacity, WSA water stable aggregates, BD bulk density, MBC microbial biomass carbon, MBN microbial biomass nitrogen, SR soil respiration, qCO_2 metabolic quotient, qMIC microbial quotient.
*p ≤ 0.05, **p ≤ 0.001, ***p ≤ 0.0001.

Table 5
Partial correlation coefficients (ρ) between the labile organic carbon fractions expressed in mg kg^{-1} (Hy-DOC, DOC, POXC, HWEC and POMC) and L g C^{-1} m^{-1} (Hy SUVA and DOC SUVA) used as dependent variable, corrected for the long term field experiments (LTEs). In addition, for comparison with the labile carbon fractions, also the correlation with TOC expressed in mg kg^{-1} has been reported. The number of samples used in the analyses was 167.

	Hy-DOC		DOC		POXC		HWEC		POMC		Hy SUVA		DOC SUVA	
Hy-DOC	1													
DOC	0.41	***	1											
POXC	0.59	***	0.41	***	1									
HWEC	0.41	***	0.24	*	0.60	***	1							
POMC	0.50	***	0.29	**	0.70	***	0.58	***	1					
Hy SUVA	-0.44	***	0.31	***	-0.08	–	-0.06	–	-0.06	–	1			
DOC SUVA	-0.37	***	0.25	*	-0.20	*	-0.20	*	-0.25	*	0.35	***	1	
TOC	0.44	***	0.33	***	0.69	***	0.52	***	0.68	***	-0.07	–	-0.16	*

Hy-DOC hydrophilic dissolved organic carbon, *DOC* dissolved organic carbon, *POXC* permanganate oxidizable carbon, *HWEC* hot water extractable carbon, *POMC* particulate organic matter carbon, *Hy SUVA* hydrophilic specific ultraviolet absorbance, *DOC SUVA* dissolved organic carbon specific ultraviolet absorbance.
*$p \leq 0.05$, **$p \leq 0.001$, ***$p \leq 0.0001$.

4.2. Effect of organic matter addition on the labile carbon fractions

High OM addition increased the concentration of the labile carbon fractions compared to low OM addition. This agricultural practice had greater impact on the labile carbon fractions than tillage, indicating the important role of organic matter addition in increasing C in soil (Table 3). Permanganate oxidizable C, Hy-DOC and POMC were more sensitive than the other fractions to OM additions (Table 2), which is in accordance with Ibrahim et al. (2013); Mirsky et al. (2008); Tatzber et al. (2015). Previous studies found that organic input increases the concentration of soil labile carbon (Benbi et al., 2015; Li et al., 2018; Pezzolla et al., 2015; Tatzber et al., 2015). Apart from direct effects of organic matter input on labile carbon through addition of organic substrates, which stimulate microbial biomass and indirect effects through provision of a suitable physical environment, OM addition can introduce external microbial populations, which also can contribute to an increase of the labile organic carbon pools (Bastida et al., 2008).

Some studies did not find effects of tillage and fertilization on the labile C fractions (Ladoni et al., 2015; Margenot et al., 2017; Sequeira and Alley, 2011). This can be due to the soil properties, the non-homogeneous distribution of plant and microbial residues, organic matter input type and quantity, environmental conditions and time of sampling. The soil type, for example, can influence the extent to which agricultural management can affect soil organic carbon. In soils with light texture, organic matter additions can have a higher beneficial effect on TOC and labile carbon than conservation tillage (Chivenge et al., 2007). Our approach, based on the selection of LTEs from different pedoclimatic zones and contrasting soil types, permitted us to identify overall trends correcting for these differences in pedoclimatic zones. Even after such corrections, we found tillage and organic matter additions to have an effect on the labile carbon fractions, and in particular on POXC and POMC.

4.3. Labile organic carbon as soil quality indicator

All the labile carbon fractions were positively correlated with each other (Table 5 and S10) and also with TOC (Tables 4 and S9), indicating that TOC is their main determinant in soil (Geraei et al., 2016; Yu et al., 2017). This suggests that dynamics of labile C fractions can be used as a proxy of TOC dynamics in soils under agricultural management. Labile carbon is, in fact, an essential starting point for the formation of more stable soil organic matter (Cotrufo et al., 2013). Of all labile carbon fractions, POXC and POMC were the two fractions that showed the strongest relationship with TOC. Moreover, POXC and POMC were the labile carbon fractions most sensitive to both tillage and organic matter management. This was true for the concentration per kg soil (Tables 1, 2 and 3) and, only for the organic matter addition management, for the concentration per unit TOC (Tables S5 and S7). By expressing POXC and POMC relative to TOC, any possible interferences from structural differences in total soil organic matter are minimized. Moreover, this normalization to TOC emphasizes generic relationships affecting labile carbon build-up in the soils, which are not directly related with organic matter additions, such as soil structure and chemical recalcitrance.

POXC was strongly correlated with labile carbon fractions that are extracted with either relatively lower (i.e. Hy-DOC, DOC, HWEC), or higher (POMC) extraction intensity (and bioavailability) than POXC.

As indicated above, POXC responded strongly to tillage and organic matter addition, and differences between sites were relatively small (Table S2). Hence, our data suggest that POXC is the most representative labile organic carbon indicator and that its dynamics are the best proxy of TOC dynamics. This agrees with findings of Hurisso et al. (2016), who found that POXC reflected soil management that aimed to increase organic matter content and stability, and suggested that POXC can be a useful indicator of C sequestration.

POXC was also the labile carbon fraction most strongly correlated to various other soil chemical, physical and biological quality parameters (Tables 4 and S9). The correlations between POXC, TOC and MBC have been attributed to specific characteristics of the extraction methods used to determine the three fractions (Geraei et al., 2016): the oxidation of POXC mimics microbial decomposition of organic matter, which is confirmed by its often positive correlation with basal respiration, substrate-induced respiration, microbial biomass and soluble carbohydrates (Weil et al., 2003).

The positive correlation between POXC and HWC, WSA and CEC, which are parameters known to be influenced by more complex organic matter (Wander, 2004), can be explained by the fact that the oxidation during the POXC reaction targets labile but also affects more recalcitrant forms of SOM. Specialized microorganisms can make use of more complex compounds (Lehmann and Kleber, 2015), which could explain the relationship between POXC and microbial biomass and activity even if permanganate reacts with more complex compounds also, as recently confirmed by Romero et al. (2018). Hence, POXC strongly relates to TOC, but also a variety of other soil quality parameters underlining its role as a multifunctional soil quality indicator. Moreover, POXC can be measured relatively cheaply and fast (Table 6). The different strengths of the correlations between labile carbon fractions and other soil quality indicators, including TOC, suggest that these fractions quantify distinct parts of the TOC with different functional characteristics.

Currently, little is known about the chemical composition and the seasonal dynamics of POXC. However, there is evidence for the sensitivity of POXC to other types of soil management beside tillage and organic matter input such as the use of cover crops, but this should be validated with further studies (Culman et al., 2012; Idowu et al., 2008). POXC was found to be linked with various soil quality indicators related to multiple soil functions, which is a very important characteristic for effective and informative soil quality indicators. In fact, POXC (named as 'Active Carbon') was included in the Comprehensive Assessment of

Table 6
Time and cost analysis for the labile carbon fractions as calculated according to the methodology applied in the current research, and the prices applied in the Chemical and Biological Laboratory of Wageningen University and Research. Relative time and costs refer to the time and money required for processing permanganate oxidizable carbon (POXC) compared to the other labile carbon fractions.

Labile carbon fraction	Relative time compared to POXC analysis	Relative analysis costs compared to POXC analysis
POXC		
DOC	3× higher	2.4× higher
Hy-DOC	3.5× higher	2.7× higher
HWEC	20× higher	2.4× higher
POMC	32× higher	1.4× higher

Hy-DOC hydrophilic dissolved organic carbon, *DOC* dissolved organic carbon, *POXC* permanganate oxidizable carbon, *HWEC* hot water extractable carbon, *POMC* particulate organic matter carbon.

Soil Health (CASH) framework, where it was recognized as a soil quality indicator besides other biological, chemical and physical parameters. The CASH is available since 2008 (Idowu et al., 2008) and is especially targeted at farmers and land managers, and widely used throughout the USA.

Recently, Fine et al. (2017) found that POXC was the best single predictor of overall soil quality measured using CASH scores. Their study included a large number (n = 930) of samples from different sites in the USA covering different pedo-climatic conditions. Still, the quantitative relationships between currently used indicators and soil functions are generally under-investigated. Therefore, establishing those relationships is of high priority and future studies should particularly address these quantitative linkages.

5. Conclusions

The labile organic carbon fractions investigated in 10 LTE fields covering a range of pedoclimatic zones within Europe appeared sensitive to soil management, showing in general increased values in reduced tillage and high organic matter input systems. Our results suggest that the different labile carbon fractions represent different soil organic carbon pools, with POXC and POMC representing pools that appear to be highly sensitive to agricultural management and less variable than the other labile carbon fractions. This makes them more suitable as soil quality indicators than the highly labile DOC and Hy-DOC, HWEC and the slowly changing TOC. In addition, concentrations of POXC and POMC are an order of magnitude higher than Hy-DOC and DOC, which strongly facilitates their measurement. Moreover, POXC is easily measured at low cost, which makes its use feasible in practice.

POXC represents a labile carbon fraction sensitive to soil management that is highly informative about total soil organic matter, nutrients, soil structure, and microbial pools and activity, parameters commonly used as indicators of various soil functions, such as C sequestration, nutrient cycling, soil structure formation and soil as a habitat for biodiversity. Therefore, we suggest measuring POXC as the labile carbon fraction in soil quality assessment schemes in addition to other valuable soil quality indicators.

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Competing interests

The authors have declared that no competing interests exist.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2018.12.008.

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